Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo 4/8/74

MALIC ACID

C19

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: April 8, 1974

THRU : Dr. Leo Friedman, Director
Division of Toxicology, HFF-150

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Malic Acid in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Malic Acid

Protocol:

Malic Acid (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Malic Acid showed only slight toxicity when injected into the air cell at 0 hours. Only at 200.00 and 100.00 mg/kg was the toxicity significantly higher than the solvent background. The LD_{50} was 230.875 mg/kg (11.544 mg/egg).

Injection into the air cell at 96 hours resulted in a much higher toxicity than observed at 0 hours. Significant ($p=0.05$) toxicity above background was obtained as low as 5.00 mg/kg and the LD_{50} calculated from the line was 8.432 mg/kg (0.4216 mg/egg).

Yolk treatment at 0 hours showed toxicity higher than solvent background at all levels between 5.00 and 200 mg/kg, while at 96 hours there was no significant toxicity between 2.5 and 50.00 mg/kg. In both cases the slope of the line was negative.

Malic Acid induced relatively few anomalies under the four conditions of test. The untreated controls had only one serious abnormality, a bird with celosomia.

Treatment via the air cell at 0 hours showed only three birds with serious abnormalities: one with exencephaly at 100 mg/kg; one with celosomia at 50 mg/kg; and one with cyclopia and ectopia cordis at 10 mg/kg. There were no abnormals in the solvent controls.

When injection was via the air cell at 96 hours there were also three birds with serious abnormalities: at 5.00 mg/kg one bird with torticollis and one bird with multiple anomalies (microphthalmia, anophthalmia, exencephaly and dysgnathia); at 2.50 mg/kg, one bird with microphthalmia. There were no abnormals in the solvent controls.

Yolk treatment at 0 hours showed only four abnormal birds: at 200 mg/kg, one bird with hypoplasia of the head and four legs; one bird with celosomia at 100, 50, and 25 mg/kg. No abnormalities occurred in the solvent controls.

Yolk treatment at 96 hours resulted in six birds with serious anomalies: 25 mg/kg, one bird with a coloboma; at 12.50 mg/kg, one bird with anophthalmia and dysgnathia; at 5.00 mg/kg, one bird with a curved maxilla and one with exencephaly; at 2.50 mg/kg, one bird with microcephaly and one bird with exencephaly. The solvent controls also had one bird with exencephaly.

The incidence of abnormalities of the head, limbs, viscera and skeleton was low for all four conditions of test, and with the exception of one dose level (50 mg/kg, air cell at 0 hours), the incidence of abnormalities was

not significantly higher ($p=0.05$) than the solvent background.

It is concluded from these data that malic acid did not show any teratogenic activity under the test conditions employed.

Table 1

Malic Acid

Air Cell at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	100	62.00*	13.00*	1.00
5.00	100.00	99	37.37*	8.08*	1.01
2.50	50.00	100	33.00	9.00*	6.00*
1.250	25.00	100	28.00	1.00	0.00
0.500	10.00	100	26.00	3.00	2.00
Water		100	20.00	0.00	0.00
Controls		360	15.55	1.66	0.55

**LC_{50} 230.875 mg/kg (11.544 mg/egg)

* Significantly different from solvent $p \leq 0.05$.

Table 2

Malic Acid

Air Cell at 96 Hours

Dose mg/egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
5.00	100.00	15	100.00*	0.00	0.00
2.50	50.00	115	100.00*	0.00	0.00
1.250	25.00	115	93.04*	6.95	2.60
0.6250	12.50	115	79.13*	6.08	1.73
0.250	5.00	115	32.17*	8.69*	3.47
0.1250	2.50	100	21.00	6.00	3.00
Water Controls		124	16.12	1.61	0.80
		360	15.55	1.66	0.55

**LC_{50} 8.432 mg/kg (0.4216 mg/egg)

*Significantly different from solvent $p \leq 0.05$

Table 3

Malic Acid

Yolk at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	70	82.85*	1.42	2.85
5.00	100.00	99	67.67*	4.04	4.04
2.50	50.00	100	73.00*	2.00	2.00
1.250	25.00	100	72.00*	4.00	1.00
0.6250	12.50	30	66.66*	10.00*	0.00
0.500	10.00	70	68.57*	4.28	1.42
0.250	5.00	30	86.66*	0.00	0.00
Water		114	43.85	0.00	0.00
Controls		360	15.55	1.66	0.55

**Slope is negative

*Significantly different from solvent $p \leq 0.05$

Table 4

Malic Acid

Yolk at 96 Hours

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
2.50	50.00	85	32.94	5.88	1.17
1.250	25.00	85	43.52	4.70	3.52
0.6250	12.50	84	53.57	5.95	2.38
0.250	5.00	85	43.52	5.88	2.35
0.1250	2.50	99	46.46	11.11*	4.04
Water		119	47.05	1.68	0.84
Controls		360	15.55	1.66	0.55

**Slope is negative

*Significantly different from solvent $p \leq 0.05$

1. Malic Acid, Lot No. 35523, Allied Chemical Co., Morristown, New Jersey.

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M., Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006.

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed. Cambridge Press, Cambridge, Appendix I.